

Antibodies to mutated citrullinated vimentin and antibodies to cyclic citrullinated peptides in juvenile idiopathic arthritis

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Abstract

Background: The goal of the study was to assess the presence of antibodies to mutated citrullinated vimentin (anti-MCV) and cyclic citrullinated peptides (anti-CCP) in patients with juvenile idiopathic arthritis (JIA) compared with patients with other juvenile onset rheumatic diseases.

Methods: The study included 56 patients who fulfilled the International League of Associations for Rheumatology (ILAR) classification criteria for JIA, and 17 control patients with other juvenile onset rheumatic diseases. Data on six core outcome variables and the Sharp score were collected for patients with JIA. Sera and synovial fluid, if available, were tested for anti-CCP and anti-MCV antibodies using a commercial enzyme-linked immunosorbent assay (ELISA).

Results: Anti-MCV antibodies were positive in 3/56 (5.4%) and anti-CCP in 1/56 (1.8%) of patients with JIA. Two out of three anti-MCV positive patients (one of them also anti-CCP positive) were found to be rheumatoid factor (RF)-positive with polyarticular disease. Within the control group, anti-MCV was positive in 4/17 (23.5%) patients, while anti-CCP positivity was not observed. No correlation between anti-MCV with anti-CCP antibody levels was found for any of the six core outcome variables or for the adapted Sharp score.

Conclusions: Our results show that antibodies targeting citrullinated proteins are not a useful diagnostic marker for JIA, but can indicate severe patterns of disease in JIA.

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Keywords: antibodies to mutated citrullinated vimentin; citrullinated proteins; juvenile idiopathic arthritis.

Introduction

Juvenile idiopathic arthritis (JIA) is the most common rheumatic disease in children (1). According to the International League of Associations for Rheumatology (ILAR) classification, JIA represents an umbrella term for a group of heterogeneous, chronic inflammatory diseases of unknown etiology (2). Among the disease forms with different onset, the long-term outcome is best in persistent oligoarthritis and worst in rheumatoid factor (RF)-positive polyarthritis, which is associated with marked disability in adulthood. With new and effective therapeutic approaches becoming widely available, it is important to diagnose and treat JIA patients as early as possible to prevent destruction of joints. Serologic testing is currently of limited value in predicting the clinical course of patients with JIA. Thus, efforts are underway to search for reliable diagnostic and prognostic biomarkers. The presence of antibodies directed against citrullinated proteins, especially the peptide mixture designated as cyclic citrullinated peptides (CCP) in the sera of patients with rheumatoid arthritis (RA), has been investigated extensively (3–9). These antibodies have reasonable sensitivity and high specificity for RA and are increasingly used in the evaluation of patients who may have RA. Recently, citrullinated vimentin was recognized as a potential native target for this family of antibodies (10). In the polymerized form, vimentin constitutes a structure of intermediary filaments, the main component of cytoskeleton in mesenchymal cells. In vivo, vimentin is usually not in a citrullinated state, but deimination of this protein occurs in macrophages that are undergoing apoptosis (11). Anti-citrullinated vimentin antibodies may then emerge as a consequence of inadequate clearance of apoptotic material in patients with RA. A mutated isoform of vimentin was identified by mass spectroscopy analysis of purified vimentin in synovial fluid samples from patients with RA. Subsequently, a human recombinant mutated isoform of vimentin, citrullinated in vitro, was used in a new commercial enzyme-linked immunosorbent assay (ELISA) for detection of the respective antibodies against mutated citrullinated vimentin (anti-MCV) (12). Studies that compared the diagnostic accuracy of anti-MCV and anti-CCP antibodies in RA found comparable sensitivities, while the specificities were higher for anti-CCP in most cases (13–19). In contrast to RA, the occurrence of antibodies targeting citrullinated proteins, especially anti-MCV antibodies in rheumatic diseases of childhood, has remained largely unknown (20–22).

The goal of our study was: (i) to assess the possible difference in serum anti-CCP and anti-MCV between patients with JIA and patients with other juvenile

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onset rheumatic diseases; (ii) to investigate the correlation between serum anti-CCP and anti-MCV with six core outcome variables and the adapted Sharp score in patients with polyarticular JIA; (iii) to determine, when possible, concentration of anti-CCP and anti-MCV in synovial fluid from patients with JIA.

Materials and methods

Patients and controls

Patients and controls were recruited prospectively during their regular follow-up visits at the Division of Clinical Immunology and Rheumatology, Children's Hospital Zagreb (Croatia) between December 2006 and December 2007. The study included 56 patients who fulfilled the ILAR classification criteria for JIA (2) and 17 control patients with other juvenile onset rheumatic diseases. Within the JIA group were 13 children with polyarticular disease, of whom only two were RF-positive. Criteria were fulfilled in 26 children for persistent oligoarticular disease, in 16 for enthesitis-related arthritis (ErA-juvenile spondyloarthropathies) and in one for juvenile psoriatic arthritis (jPsA). One child with persistent oligoarticular disease had inactive systemic-onset disease. The control group included 12 patients with systemic lupus erythematosus (SLE), two patients with post-traumatic synovial cysts, one patient with mixed connective tissue disease (MCTD), one with fibromyalgia, and one with Osgood-Schlatter disease.

Clinical assessment

Patients were diagnosed and treated in the absence of any information on their serologic status concerning anti-MCV or anti-CCP antibodies. Clinical data, serum samples and paired synovial fluid samples (when available) were taken during the first 12 months of the disease (early onset) in 14 patients. For the remaining patients, samples were collected regardless of disease status. Paired synovial fluid samples were obtained in 12 patients. Standard six core outcome variables, erythrocyte sedimentation rate (ESR), physician global assessment visual analogue scale (VAS-MD), patient global assessment visual analogue scale (VAS-Pt), Children's Health-Assessment Questionnaire disability index (CHAQ-DI), the number of active joints and joints with limited range of motion (ROM), described in detail elsewhere (23, 24), were assessed for each patient with JIA. CHAQ-DI was validated previously in Croatian children (25). Standard definitions of flare-up, improvement and remission of disease were used in assessing the disease status of JIA patients (23–27). When available, the adapted Sharp index was calculated (28). Among the patients with JIA, nine with the most severe disease were undergoing therapy with anti-tumor necrosis factor- α (anti-TNF α). The study was approved by Children's Hospital Zagreb Ethics Committee, and informed consent was obtained from the parents of all patients and controls.

Laboratory analyses

Blood was collected from all patients, immediately centrifuged and serum was aliquoted. C-reactive protein (CRP) and ESR were measured in fresh samples. Aliquots of serum and synovial fluid for anti-MCV and anti-CCP antibody concentrations were stored at -20°C until analysis.

Serum and synovial antibody concentrations were measured with an anti-MCV ELISA (Orgentec, Mainz, Germany), and second-generation anti-CCP2 ELISA test (Euroimmun, Luebeck, Germany). The manufacturer's cut-off thresholds of 20 U/mL and 5 RU/mL for anti-MCV and anti-CCP antibodies, respectively, were used. Dilution protocols for serum and synovial fluid were identical.

Statistical analysis

Each distribution was tested for normality using the Kolmogorov-Smirnov test prior to further statistical analysis. The difference in anti-CCP and anti-MCV antibody concentrations between groups was tested with the Mann-Whitney rank sum test. Correlation between antibody concentrations and the six core outcome variables and Sharp score was tested with Spearman rank correlation test. Bonferroni adjustment was applied for multiple testing. Statistical analysis was performed using MedCalc[®] statistical software (MedCalc 10.1.8.0, Frank Schoonjans, Mariakerke, Belgium). A p -value < 0.05 was chosen for statistical significance.

Results

Demographic data of patients with JIA and the control group are presented in Table 1. JIA patients, classified into disease subtypes according to ILAR criteria and their clinical and biochemical characteristics, are listed in Tables 2 and 3.

Median anti-MCV and anti-CCP antibody concentrations were within the normal range in both groups, though significantly higher in the control group compared to patients with JIA (Table 4). Among patients with JIA, those with disease flare had higher anti-MCV and anti-CCP antibody concentrations. This difference was statistically significant (Table 5).

Anti-MCV antibodies were positive in 3/56 (5.4%) and anti-CCP in 1/56 (1.8%) of patients with JIA. Two out of three patients positive for anti-MCV were found to be RF-positive with polyarticular disease. Their anti-MCV concentrations were substantially higher compared to the third anti-MCV positive patient with ErA. However, only one of these patients was also anti-CCP-positive. No correlation between anti-MCV antibody concentrations and any of the six core outcome variables or with the adapted Sharp score was found. For anti-CCP, weak correlation was found with ESR ($r=0.313$, $p=0.030$) and CHAQ-DI ($r=0.346$,

Table 1 Demographic data of patients with JIA and controls.

	JIA patients (n=56)	Control group (n=17)
Age, years ^a	11 \pm 4	16 \pm 3
Female, %	73	71
Age at onset of disease, years ^a	7.9 \pm 4.9	13.2 \pm 2.4
Disease duration, years ^a	3.4 \pm 3.0	2.7 \pm 2.0

^aMean \pm SD. JIA, juvenile idiopathic arthritis; SD, standard deviation.

Table 2 JIA subtypes according to ILAR (n=56).

JIA subtype	n
Oligoarticular (persistent)	26
ErA	16
Polyarthritis RF-positive	2
Polyarthritis RF-negative	11
Psoriatic arthritis	1

JIA, juvenile idiopathic arthritis; ILAR, International League of Associations for Rheumatology; ErA, enthesitis-related arthritis; RF, rheumatoid factor.

Table 3 Clinical and biochemical characteristics of patients with JIA (n=56).

Characteristics	Value
Patients with disease flare, n	7
RF positive patients, n (%)	3 (5.4)
CCP positive patients, n (%)	1 (1.8)
CRP, mg/L	0.6 (0.3–2.8) ^a
ESR, mm/h	10.0 (7.0–13.3) ^a
VAS-MD	1.7 ± 1.27 ^b
VAS-Pt	1.4 ± 1.28 ^b
CHAQ-DI (n=44)	0.06 (0.00–0.45) ^a
Number of active joints	1 (0–5) ^c
Number of joints with limited ROM	0 (0–2) ^c
Sharp index (n=12)	0.58 ± 0.514 ^b

^aMedian (IQ range); ^bmean ± SD; ^cmedian (min-max). JIA, juvenile idiopathic arthritis; RF, rheumatoid factor; CCP, cyclic citrullinated peptides; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; VAS-MD, physician global assessment visual analogue scale; VAS-Pt, patient global assessment visual analogue scale; CHAQ-DI, Children's Health Assessment Questionnaire disability index; ROM, range of motion.

p=0.023). When adjusted for multiple comparisons, the correlations were not significant any more. No statistically significant difference was found between anti-CCP or anti-MCV concentrations in patients with JIA with respect to therapy with anti-TNF α (p=0.815 and p=0.228, respectively).

Within the control group, anti-CCP positivity was not observed, while the anti-MCV positivity rate was 4/17 (23.5%). Out of the four patients positive for anti-MCV, three had SLE and one had MCTD. Anti-MCV

concentrations were increased substantially in two of them. Correlation between anti-MCV and anti-CCP was more evident in the control group (r=0.547, p=0.029) compared with JIA patients (r=0.345, p=0.010).

Synovial fluid was obtained from 12 patients. Mean synovial antibody concentrations were 0.7 ± 0.66 for anti-CCP and 5.4 ± 2.09 for anti-MCV. Serum and synovial fluid anti-CCP and anti-MCV antibody concentrations showed no correlation (r=0.397, p=0.234 and r=0.496, p=0.099, respectively).

Discussion

The diagnostic and prognostic performance of antibodies targeting citrullinated peptides in RA has recently been investigated extensively (5–9). In order to define a native target for this family of antibodies, reactivity to a number of proteins in their citrullinated form has been investigated in patients with RA. This includes α - and β -fibrin chains (29), collagen type I (30), fibronectin (31) and α -enolase from granulocytes invading RA-synovial tissue (32). Among these proteins, vimentin has emerged as a good potential target since it has recently been identified as an antigen for well-known highly RA-specific Sa antibodies (10). The relatively low prevalence of anti-citrullinated vimentin antibodies in RA encouraged the search for other possible modifications of vimentin, apart from citrullination, that influence the antigenic properties of this protein. Recently, it was shown that exposure to oxidative stress induces mutations of glycine to arginine residues within the vimentin DNA. Although it was shown that mutated vimentin is also citrullinated in the synovial fluid of patients with RA, mutations in vimentin represent an independent trigger of antigenic properties (12). Authors using commercial ELISA methods for the detection of anti-MCV reported sensitivities and specificities of these antibodies for RA to range from 69.5% to 84%, and 87% to 98%, respectively (12–14, 17, 19, 33–35). In contrast to RA, anti-CCP antibodies appear to be rarely present in

Table 4 Anti-MCV and anti-CCP antibody concentrations in patients with JIA and controls.

	JIA (n=56)	Control group (n=17)	p-Value
Anti-CCP, RU/mL	0.5 (0.3–0.7) ^a	0.7 (0.5–2.3) ^a	0.006
Anti-MCV, U/mL	6.8 (4.9–9.1) ^a	14.8 (8.1–21.1) ^a	<0.001

^aMedian (IQ range). Anti-MCV, antibodies against mutated citrullinated vimentin; anti-CCP, antibodies against cyclic citrullinated peptides; JIA, juvenile idiopathic arthritis.

Table 5 Anti-MCV and anti-CCP antibody concentrations in patients with JIA with and without disease flare.

	JIA with disease flare (n=7)	JIA without disease flare (n=49)	p-Value
Anti-CCP, RU/mL	0.7 (0.5–2.3) ^a	0.5 (0.3–0.6) ^a	0.046
Anti-MCV, U/mL	8.4 (8.3–12.8) ^a	6.5 (4.7–9.0) ^a	0.041

^aMedian (IQ range). Anti-MCV, antibodies against mutated citrullinated vimentin; anti-CCP, antibodies against cyclic citrullinated peptides; JIA, juvenile idiopathic arthritis.

patients with JIA (20, 21, 36, 37), while data on the presence of anti-MCV in JIA is seldom reported (38).

The first objective of this study was to assess the presence of anti-CCP and anti-MCV antibodies in sera of patients with JIA compared to patients with other juvenile onset rheumatic diseases. The observed anti-CCP positivity (1.8%) in our group of JIA patients is consistent with that reported by Machado et al. (36). These investigators found anti-CCP antibodies in 2% of children suffering from JIA, while Avcin et al. (20) reported 1.8% anti-CCP positivity. The prevalence of anti-MCV positivity in our JIA patients was higher (5.4%), although not statistically significant, compared to anti-CCP. The previously reported almost exclusive association of anti-CCP positivity with RF-positive polyarthritis JIA subtype was also confirmed in our study (20, 21, 36, 37). According to limited amount of data, the same association is valid for anti-MCV reactivity in patients with JIA, which was also confirmed in this study (38). RF-positive polyarticular JIA subtype has been taken as being more severe and represents early onset of RA. This fact, together with the observation that our anti-MCV-positive patients presented with later onset of disease (11, 13 and 14 years), supports the conclusion of previous studies that antibodies to citrullinated proteins could be the marker of a JIA subgroup with increased potential to progress to typical adult RA.

Although the concentrations of both anti-MCV and anti-CCP in the subgroup of JIA patients with flare appears to be higher compared to patients without flare, the overlapping results and a small number of patients with flare ($n=7$) make this difference questionable ($p=0.041$ and $p=0.046$, respectively). Additionally, the finding that the concentrations of both antibodies were within the reference interval does not support the significance of this difference. Therefore, the association of antibodies targeting citrullinated proteins with disease severity cannot be confirmed. According to our results, the specificity was lower for anti-MCV (76.5%) than for anti-CCP (100%) antibodies in patients with JIA, which is consistent with the results of Bizzaro et al. (19) who found significantly lower diagnostic accuracy in RA for anti-MCV in comparison to the methods that use original synthetic CCP. These authors suggested the importance of the influence of antigen source as well as antigen preparation in determining method performance. Thus, specificity can be affected by the presence of proteins or contaminating peptide sequences. In our study, anti-MCV positivity was found in 23.5% patients with other juvenile onset rheumatic diseases (primarily SLE). This observation was very similar to that reported by Sghiri et al. (18) who found 21 anti-MCV positive patients out of 59 patients with SLE. They suggested two explanations for this observation: one concerns citrullination of vimentin as a post-translational modification not specific for RA, while the other concerns citrulline-independent anti-MCV reactivity. According to our results, we can confirm previous observations that in JIA patients both antibody types are of acceptable specificity but with poor sensitivity.

Performance of both anti-MCV and anti-CCP is better in RF-positive polyarticular JIA subtype.

The second objective of our study was to explore the correlation of serum anti-MCV and anti-CCP antibody concentrations with six core outcome variables and the Sharp score in patients with JIA. No correlation between anti-CCP and anti-MCV concentrations with any of the six core outcome variables or adapted Sharp score was observed. Although Guseinova et al. (39) reported positive correlation between anti-MCV antibodies and markers of inflammation CRP ($p<0.05$) and serum amyloid A (SAA, $p<0.01$) in patients with JIA, we failed to confirm these finding in our study. However, since no correlation coefficient was given in their study, the strength of the correlation is unknown. In a study performed on adult patients with early arthritis, Ursum et al. (16) found very low correlation between anti-MCV concentrations and CRP, ESR and swollen joint count at baseline and at 1 and 2 years of follow-up. However, anti-MCV antibodies were associated with more severe RA disease, as measured by disease activity score (DAS) 28, ESR, and swollen joint count over time. Similar to our study, no correlation was found between anti-MCV concentrations and tender joint count or general health determined using VAS. However, in the study of Bang et al. (12) performed on 42 RA patients, anti-MCV antibodies were significantly correlated with DAS28 ($r=0.5334$, $p=0.003$), while anti-CCP antibodies failed to show significant correlation. According to our observations, together with findings by others, anti-MCV and anti-CCP antibodies do not appear to be useful as a marker of disease activity, at least in our JIA patient cohort.

Recent RA synovial tissue studies have shown that the high specificity of anti-citrullinated protein antibodies for RA appear to be the result of abnormal humoral response rather than representing disease-specific expression of citrullinated proteins. It appears that the presence of citrullinated proteins in inflamed synovium is not specific for RA, but rather may be an inflammation-associated phenomenon (40). Independent of disease duration, synovitis in patients with anti-CCP-positive RA differs from that in patients with anti-CCP-negative RA, notably with respect to increased infiltrating lymphocytes and higher rate of local joint destruction (41). Caspi et al. (42) suggested that determination of anti-CCP antibodies in synovial fluid can have additive diagnostic value in anti-CCP seronegative RA patients. Some of the patients in their RA cohort demonstrated positive synovial fluid anti-CCP, despite the absence of these antibodies in sera. We failed to confirm the presence of anti-CCP or anti-MCV antibodies in synovial fluid from our patients with JIA. To our knowledge, this is the first study of anti-CCP and anti-MCV antibodies in synovial fluid of patients with JIA.

The major limitations of our study are the low number of control samples ($n=17$) and RF-positive polyarticular patients ($n=2$), lack of stratification of patients based on disease duration (early vs. long-term), and lack of the follow-up values for anti-MCV antibodies following 1 year of treatment.

In conclusion, our results are consistent with previous reports showing that antibodies targeting citrullinated proteins indicate JIA patients with severe clinical subtypes of disease that might progress further to an adult RA phenotype. In addition, anti-MCV seems to be more sensitive but less specific compared with anti-CCP antibodies. We could not confirm the usefulness of anti-MCV or anti-CCP antibodies as markers of disease activity. The putative association of antibody concentrations with disease flare in JIA patients needs to be confirmed in a larger prospective follow-up study. Also, high anti-MCV antibody concentrations in patients with SLE require further clarification in a larger group of patients.

Conflict of interest statement

There are no conflicts of interest.

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